Rhodora

JOURNAL OF THE

NEW ENGLAND BOTANICAL CLUB

Vol. 63 | October, 1961 | No. 754

THE CHROMOSOMES OF PODOPHYLLUM PELTATUM

GABRIELE N. MÜHLING AND G. B. WILSON[1]

Podophyllum peltatum L., commonly known as May Apple, Mandrake or Pomme de Mai, represents one of the best sources of cytological material for class purposes. It is found in eastern North America, east from a line drawn from Minnesota to Texas, including southern Ontario and Quebec. Representatives of the genus also occur in eastern Asia (Fernald, 1950). Large amounts can be collected easily and the sporogenous material is normally found to be in meiosis for about a two week period. Good cytological preparations can be made by simple techniques. The early stages of meiosis especially pachytene can be fixed and stained giving a clarity that equals maize. The chromosomes are large and few in number.

The advantages of the material do not seem to be recognized very generally probably because the cytology has not been described in any detail since 1926 when Kaufmann published his general description of the karyotype and meiosis. It therefore seemed worthwhile reporting and illustrating the work from our own collections of this material.

MATERIALS AND METHODS

Flower buds of *Podophyllum peltatum* L. were collected in the Oakland and Ingham Counties of Michigan. Collections

[1]Department of Botany, Montana State University, Missoula, Montana; and Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan.

The authors wish to thank Mr. P. G. Coleman for his preparations of the illustrations.

of anthers from these areas have been made yearly for the past six years. The most extensive collections were made in 1954 and 1959 and the main part of the data for this paper were obtained from these. Buds from the former year were fixed in the field in 3 parts absolute ethyl alcohol and 1 part glacial acetic acid and from the latter year in a 6:3:2 mixture of absolute methanol, chloroform and propionic acid (Piennar, 1955) and then placed under vacuum as soon as possible. Microspore mother cells of anthers were prepared for analysis mostly by staining by the Feulgen technique after a 9-10 minute hydrolysis in 1 N HCl at 60° C. Each anther was carefully squashed on a slide to separate the sporogenous tissue from the tapetum. Slide preparations were dehydrated in a 9:1 mixture of tertiary butyl alcohol and absolute ethyl alcohol overnight and then mounted in diaphane. Some material was stained with aceto-carmine. Fixed anthers were macerated in 1 N HCl at 60° C for 5 minutes prior to staining. Before dehydration, excess carmine was removed from preparations by running a drop of 45% acetic acid under the cover slip. If this is not done the cytoplasm becomes dark, often obscuring the division figures.

DISCUSSION

1. *Karyotype:* Our observations of a haploid number of six chromosomes in *P. peltatum* are in agreement with those earlier reported by Litardière (1921) and Kaufmann (1926). The kinetochores are found in three positions — each type appearing twice in the karyotype. In two chromosomes the kinetochores are in a near-median position; in two, in a submedian position and in two, in a subterminal position. The relative lengths of the arms of the chromosomes measured at anaphase II in the above order are: approximately 1:1; 1:2; and 1:18. For convenience the three types shall be called A, B, and C respectively. One chromosome of each of the three types has a satellite. It appears on the long arm in types B and C (Plate 1262, fig. 7, 8 and 9). It is not possible to determine which arm contains the satellite in type A because of their near identical length and the lack of any other morphologically distinguishing characteristic

(Plate 1262, fig. 9). In some details this description of the satellites does not agree with that of Kaufmann (1926) which may be because of the difficulty in detecting them or because a different population was sampled. A number of

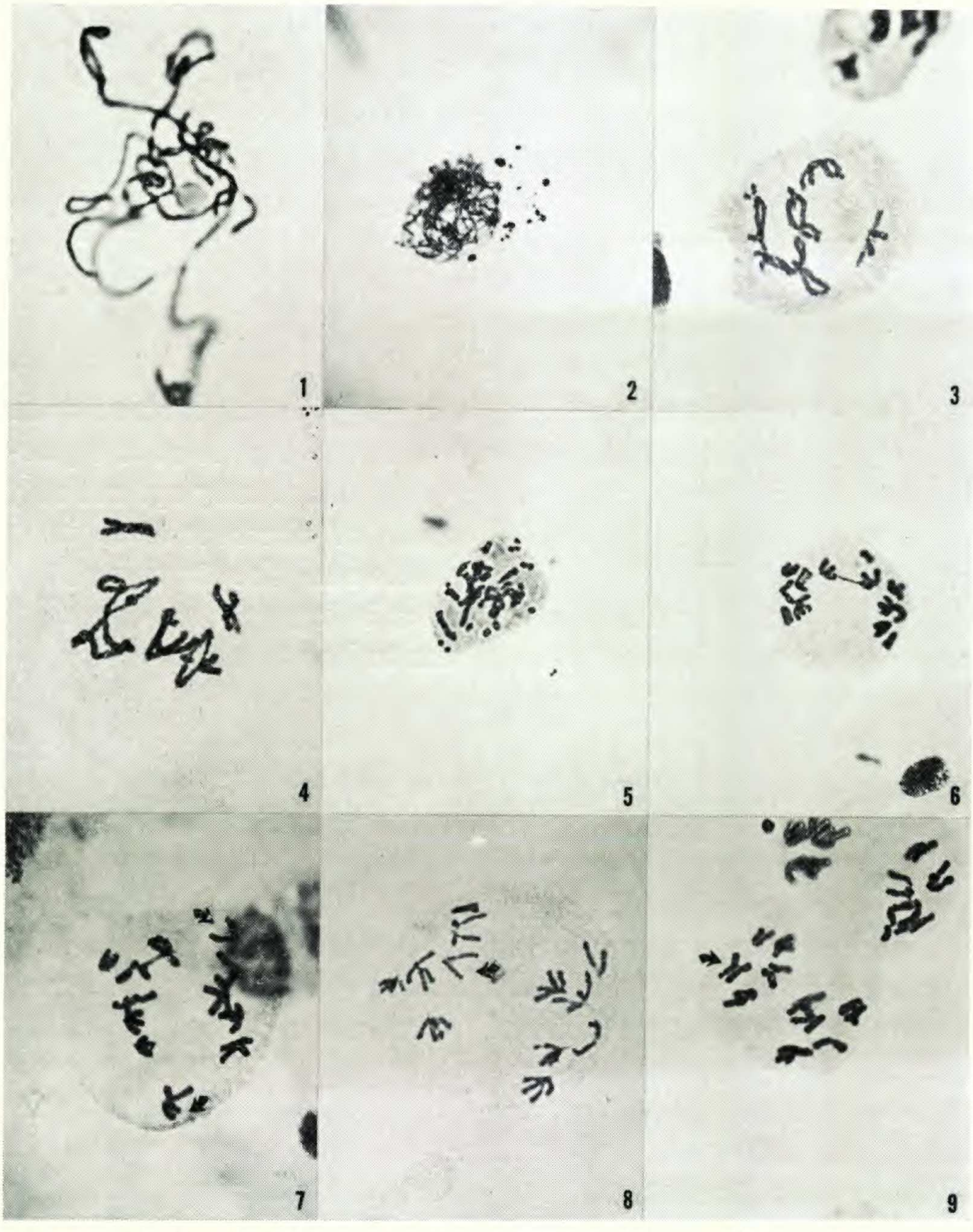

PLATE 1262. Fig. 1. Pachytene showing nucleolar attachment. Fig. 2. Zygotene with DNA blobs. Fig. 3. Diakinesis and faint outline of nucleolus. Fig. 4. Interlocking chromosomes. Fig. 5. Probably a metaphase I with numerous fragments. Fig. 6. First anaphase bridge and fragment. Fig. 7-9. Anaphase I. Arrows point to chromosomes with satellites.

peculiarities are noted in the occurrence of these satellites. First of all we did not observe them in somatic anaphases when they generally do appear in material which has them. Furthermore frequently a satellite is only visible in one chromatid of a first division anaphase chromosome, its partner having no indication of this structure whatsoever (Plate 1262, fig. 9).

The total length of the complement of six chromosomes of this species is about 60 microns. The lengths of each of the chromosomes expressed in terms of the total length of the complement is approximately as follows: the median attached chromosome, 20% ; submedian 17% ; and subterminal, 13%.

2. *General description of meiosis:* The key stages are shown in the figures in Plate 1263. They are consistent with the generalized textbook descriptions. Pachytenes are particularly good. In cells where they are sufficiently spread out, it is possible to count the number of chromomeres in a chromosome. The interkinesis between the two meiotic divisions is well marked, sometimes being so complete that nucleoli appear although this seems to be somewhat unusual. Cytokinesis does not occur until the end of second division so that diads are sometimes confused with binucleate tapetal cells since they are somewhat similar in size and shape.

A number of special features of the material deserve some comment:

a. The nucleolus: In somatic tissue the number of nucleoli appears to vary from one to three. So far we have failed to determine with which chromosomes they are associated. In meiosis there is consistently a single nucleolus, which is associated with one of the two subterminally attached chromosomes. Although the satellite can not be seen all of the time, it is assumed that the nucleolar association is with the chromosome possessing the secondary constriction. The nucleolar organizer region appears to be in the proximal third of the long arm and is not associated with any obvious secondary constriction. In any case, the three secondary constrictions noted do not seem to be concerned with nucleolar formation. In some plants such as maize (McClintock, 1934) the nucleolus develops from a definite organizing region in the area of the secondary constriction.

b. Some microsporocytes that appear to be between zygotene and pachytene frequently show a large number of Feulgen positive bodies (Plate 1262, fig. 2). Similar observa-

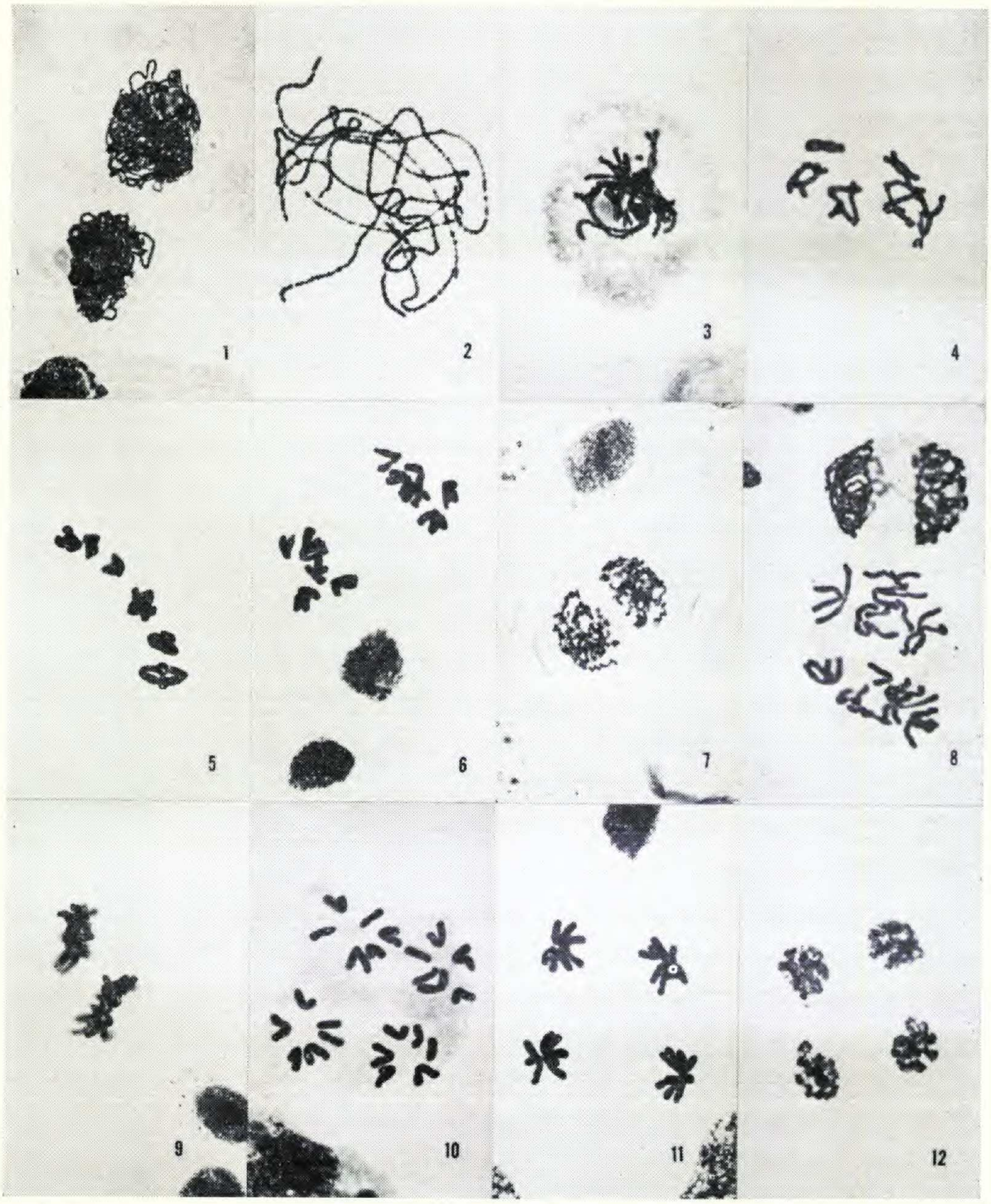

PLATE 1263. Stages of meiosis in *Podophyllum peltatum*. Fig. 1. Zygotene; 2. Pachytene; 3, Diplotene; 4, Diakinesis; 5, Metaphase I; 6, Anaphase I; 7, Telophase I; 8, Prophase II; 9, Metaphase II; 10, Anaphase II; 11, Late anaphase II; 12, Telophase II.

tions have been made in *Trillium* (Sparrow & Hammond, 1947) and *Lilium* (Cooper, 1952 and Takats, 1959). Sparrow and Hammond (1947) suggest that this represents a transfer of nuclear DNA to the cytoplasm. Similarly Cooper suggests that this represents movement of DNA from the tapetum to the locules and ultimately into the nuclei of the microsporocytes. After an extensive examination of this phenomenon, Takats (1959) concludes that there is no evidence for transfer of DNA to the microsporocytes from the tapetum during this stage in meiosis. He suggests that the extrusion may be caused by such factors as injury to the anthers at time of harvest and type of fixative used. We are inclined to take the view that they represent an abnormality of some sort and doubt that such cells proceed through meiosis. Occasionally observations such as the configuration shown in Plate 1262, fig. 5 in which there is very considerable fragmentation suggests the possibility of a relationship between the abnormal zygotenes and pachytenes and the cells showing the chromatin pieces. Similar extreme fragmentation beginning at first metaphase is reported by Gentcheff and Gustafsson (1940) in an apomict, *Hieracium robustum*.

c. Populations examined so far quite consistently have revealed a first anaphase bridge and fragment suggesting heterozygosity for an inversion in what appears to be the median attached chromosome (Plate 1262, fig. 6).

d. Chromosome interlocking as illustrated in Plate 1262, fig. 4 also occurs occasionally.

3. *Chiasma frequency:* Counts of the chiasma frequency at diakinesis were made in samples of both the 1954 and 1959 material. The average of the former year is $10.9 \pm .83$ and the latter year $11.6 \mp .86$, the difference not being significant. Some terminalization takes place between early diakinesis and first metaphase but the number of chiasmata is not notably decreased till the beginning of first anaphase.

4. *Tapetal cells:* It is quite common for the tapetal cells in plants to show considerable aberration with reference both to mitosis and number of chromosomes (D'Amato,

1952). *P. peltatum* is particularly good material for illustrating aberrations. Some of the more striking ones are shown in Plate 1264. The tapetal cells begin division concurrent with the onset of meiosis and these first divisions are generally normal. By diplotene, tapetal divisions begin to show abnormalities in the form of scattered metaphase configurations and the frequency of divisions is on the increase. Between first metaphase and second prophase, the rate of mitosis in the tapetum seems to reach its peak and also the degree of aberration has increased considerably. Polyploidy is very common now, often to the octoploid level and in a few cases probably much higher. In some apparent-

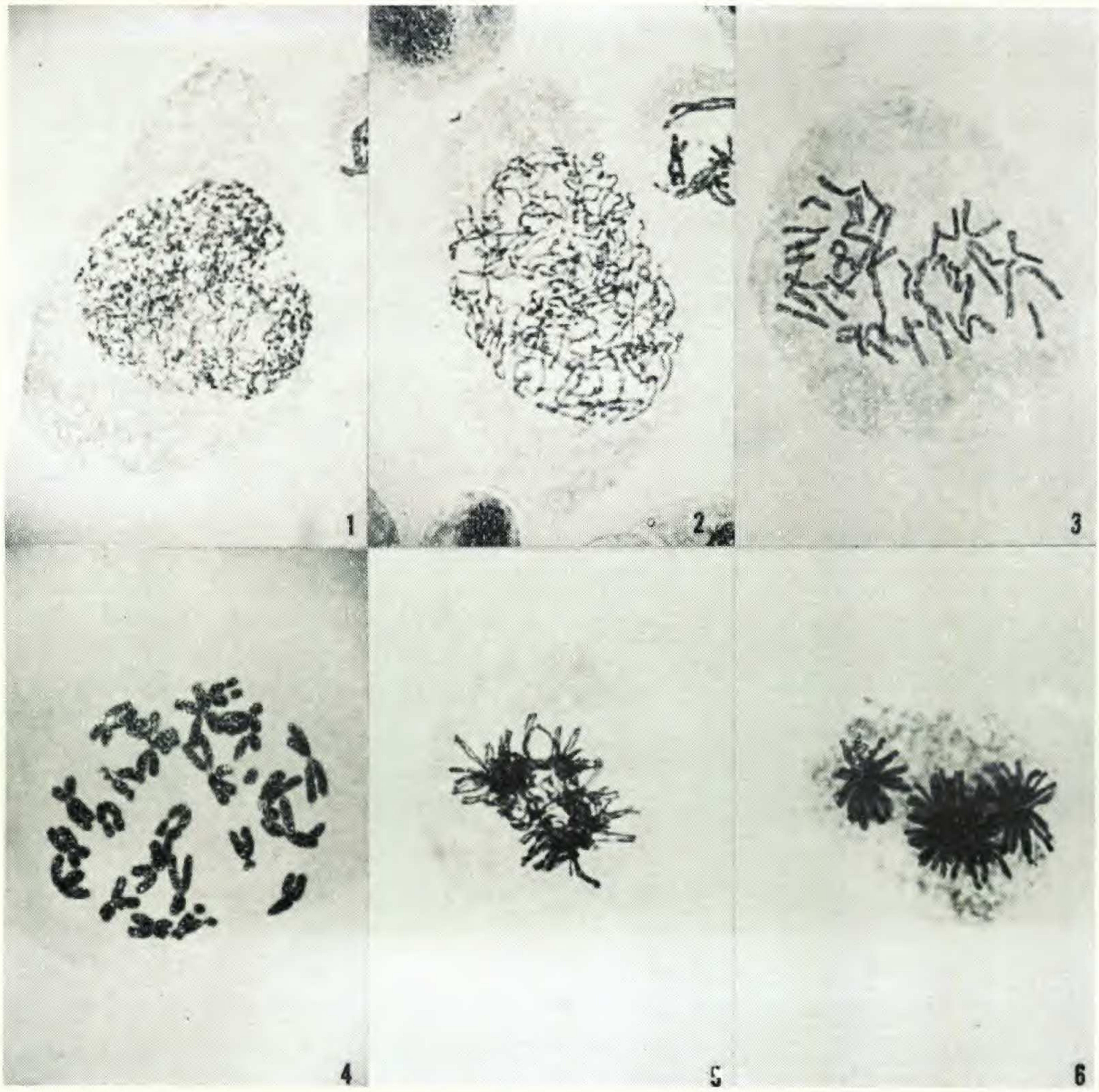

PLATE 1264. Abnormal stages in mitosis of the tapetum. Fig. 1-2. Polyploid prophase. Fig. 3. Polyploid metaphase. Fig. 4. "Scattered" metaphase. Fig. 5. Metaphase. Fig. 6. Tri-polar anaphase.

ly clear cases the number is not an exact multiple of the basic number but one or more chromosomes is missing. Other characteristics are multinucleate cells with varying numbers of chromosomes in the different nuclei, star metaphases, star anaphases with three or more centers of aggregation. By the time tetrads are formed there is a definite decrease in the tapetal divisions and an over-all multinucleate condition exists in the interphases of this tissue. Indeed almost all of the mitotic aberrations which have been associated with chemical treatments or described for cancer cells are characteristically found in the tapetal cells of this plant.

The question may be asked whether the abnormalities of the tapetum arise as a result of its function. In her studies on *Solanum tuberosum* Avanzi (1950) suggests that the tapetum definitely has a nutritive function and that the abnormalities especially in chromosome number, occur at the time when the sporogenous tissue needs its greatest food supply. Taylor (1959) working on *Lilium longiflorum* came to the conclusion that the primary function of the tapetal layer is to secrete material for wall formation of the microspores. Further work needs to be done on this intriguing phenomenon in the tapetum to determine its cause.

SUMMARY

The above account describes microsporogenesis in anthers of *Podophyllum peltatum* L. and the concurrent mitotic activity of the tapetum. Some peculiarities related to these events are also mentioned.

AVANZI, M. G. 1950. Endomitosi e mitosi a diplocromosomi nello sviluppo delle cellule del tappeto di *Solanum tuberosum* L. Caryologia **2**: 205-222.

COOPER, D. C. 1952. The transfer of desoxyribonucleic acid from the tapetum to the microsporocytes at the onset of meiosis. Amer. Naturalist **86**: 219-229.

D'AMATO, F. 1952. Polyploidy in the differentiation and function of plant tissues and cells. Caryologia **4**: 311-358.

FERNALD, M. L. 1950. Gray's Manual of Botany, eighth edition. American Book Company.

GENTCHEFF, G. AND A. GUSTAFSSON. 1940. The balance system of meiosis in *Hieracium*. Hereditas **26**: 209-249.

KAUFMANN, B. P. 1926. Chromosome structure and its relation to the chromosome cycle. II. *Podophyllum peltatum*. Amer. J. Bot. **13**: 355-363.

LITARDIÈRE, R. DE. 1921. Remarque au sujet de quelques processus chromosomiques dans les noyaux diploidiques du *Podophyllum peltatum* L. Compt. Rend. Acad. Sci. Paris **172**: 1066-1068.

MCCLINTOCK, B. 1934. The relation of a particular chromosomal element to the development of the nucleoli in *Zea mays*. Z. Zellforsch. **21**: 294-328.

PIENNAR, R. DEV. 1955. Combinations and variations of techniques for improved chromosome studies in the Gramineae. Jour. South African Bot. **21**: 1-8.

SPARROW, A. H. AND M. R. HAMMOND. 1947. Cytological evidence for the transfer of desoxyribose nuclei acid from nucleus to cytoplasm in certain plant cells. Amer. J. Bot. **34**: 439-445.

TAKATA, S. T. 1959. Chromatin extrusion and DNA transfer during microsporogenesis. Chromosoma **10**: 430-453.

TAYLOR, J. H. 1959. Autoradiographic studies of nucleic acids and proteins during meiosis in *Lilium longiflorum*. Amer. J. Bot. **46**: 477-484.

A LIST OF ALGAE FROM SELECTED AREAS IN MASSACHUSETTS[1]

EDGAR E. WEBBER

For the past thirty years, work on the flora of Worcester County has been centered upon vascular plants. Numerous references may be found elsewhere. Recently, attention has been directed to other forms of plant life (1, 3). However, little is known concerning the algae of the county. In 1899 Stone (6) included a list of algae found in Lake Quinsigamond as part of a floristic study of that area. Auyang (2) has recently completed a survey of the algae in Lake Quinsigamond. These two works appear to be the only ones to date dealing with the algal flora of the county, but they both are restricted to only one location.

The writer (7) has just completed an ecological study of the algal populations in eleven selected stations in Worcester

[1]The taxonomic list, slightly modified, from a thesis, "The Ecology of Some Attached Algae in Worcester County, Massachusetts," presented to the Faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Master of Science.